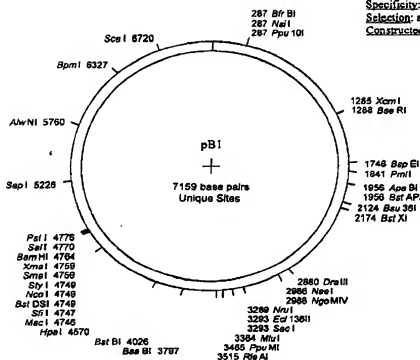


pB1

Alias: pAS2DD  
Application: 2HY (hair)  
Backbone:  
Specificity:  
Selection: ampicillin  
Constructed by:



Oligo 160

gagagtagtaacnaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT AT

Sfi I	Sma I	BamH I	Sal I	Pst I
G	GCC	ATG	GAG	GCC
		CCG	GGG	ATC
		CGT	CGA	CCT
			GCA	GCC

Nco I

Oligo 161

AAG CTA ATT ccggcggaattctatg

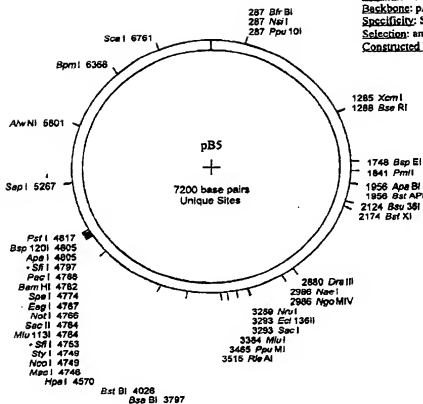
Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'

Oligo 161 5' CATAAGAAATTCGCCCG 3'

FIGURE 1

pB5<sup>2</sup>

Alias: pAS2DDNS1  
Application: 2HY (bait)  
Backbone: pAS2DD  
Specificity: Sfi non-oriented  
Selection: ampicillin  
Constructed by: SW



Oligo 160

**gagagtgtatcaaaaggtc** AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

**Sfi I** **Sac II** **Spe I** **Bam HI**  
GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C  
**Neo I** **Not I**

**STOP** **Sfi I** **Pst I**  
TT AAT **TAA** GGG CCA CTG GGG CCG CTC GAC CTG CAG CCA

**Pac I**

Oligo 161

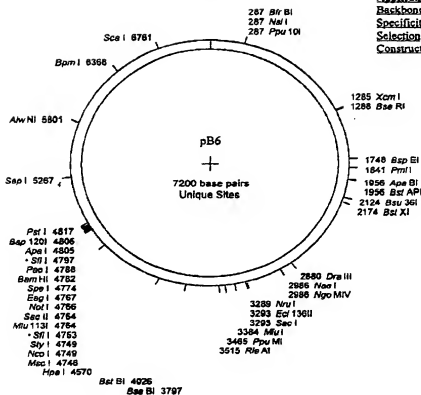
AGC TAA TT **ccggcggaatttcctatg**

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'

Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 2

pB6<sup>3</sup>



Application: 2HY (bait)  
 Backbone: pAS2DD  
 Specificity: Sfi oriented  
 Selection: ampicillin  
 Constructed by: SW

Oligo 160

gagagtagtaacaagggtcAAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I Sac II Spe I Bam HI  
 GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C  
Not I  
Neo I

STOP Sfi I Apa I Pst I  
 TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA  
Pac I

Oligo 161

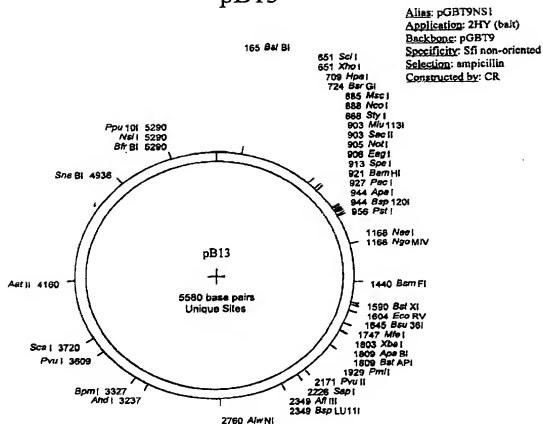
AGC TAA TT ccggcggaattctatg

Oligo 160 5' GAGAGTAGTAACAAAGGTC3'

Oligo 161 5' CATAAGAAATTCGCCCGG3'

FIGURE 3

# pB13



## Oligo 160

**gagagtagtaacaaggctc** AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

		<u>Sfi I</u>		<u>Sac II</u>		<u>Spe I</u>		<u>Bam HI</u>					
GCC	ATG	GCC	GCA	GGG	GCC	GCG	GCC	GCA	CTA	GTG	GGG	ATC	C
<u>Neo I</u>						<u>Not I</u>							
		<u>STOP</u>		<u>Sfi I</u>				<u>Pst I</u>					
TT	AAT	<b>TAA</b>	GGG	CCA	CTG	GGG	CCC	CTC	GAC	CTG	CAG	CCA	
		<u>Pac I</u>											

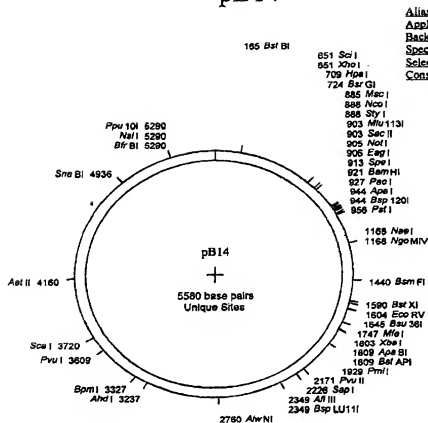
## Oligo 161

AGC TAA TT **ccggcggaattcttag**

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'  
Oligo 161 5' CATAAGAAATTCGCCCCG 3'

FIGURE 4

# pB14<sup>5</sup>



Alias: pGBT9NS2  
 Application: ZHY (best)  
 Backbone: pCBT9  
 Specificity: Sfi oriented  
 Selection: ampicillin  
 Constructed by: CR

## Oligo 160

gagagtagtaacaaagggtc AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I      Sac II      Spe I      Bam HI  
 GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C  
 Nco I      Not I

STOP      Sfi I      Apa I      Pst I  
 TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA  
 Pac I

## Oligo 161

AGC TAA TT ccggcggaatttctatg

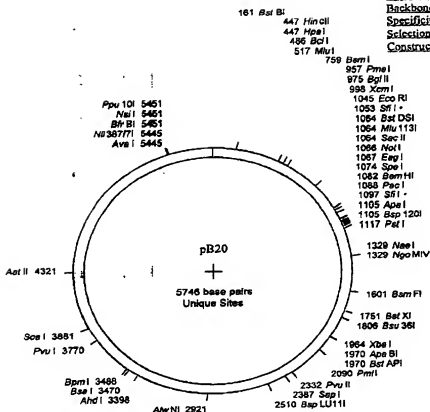
Oligo 160 5' GAGAGTAGTAACAAAGGTC3'

Oligo 161 5' CATAAGAAATTCGCCCCG3'

FIGURE 5

# pB20<sup>6</sup>

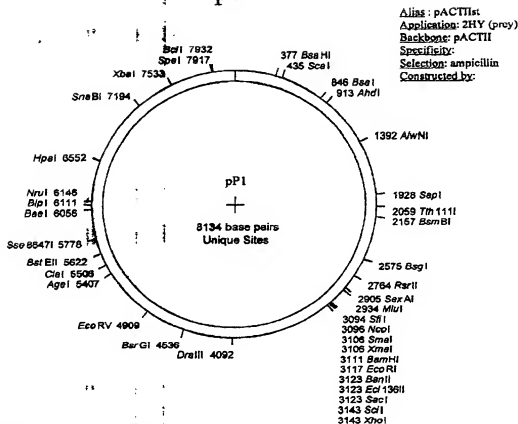
Alias: pLcx10NS2  
 Application: ZHY (bait)  
 Backbone: pLcx10 (pB9)  
 Specificity: Sfi-oriented  
 Selection: ampicillin  
 Constructed by: LD



<u>EcoRI</u>	<u>SfiI</u>	<u>NotI</u>	<u>SpeI</u>	<u>BamHI</u>
GAA TTC	GGG GCC GGA CCG GCC	GCG GCC	GCA CTA GTG	GGG ATC C
Sac II				
STOP				
TT AAT TAA	GGG CCA CTG GGG CCC	CTC GAC	CTG CAG	
<u>PacI</u>	<u>SfiI</u>	<u>PstI</u>		

FIGURE 6

# pP1



## ABS1

cgtttggaaatcaactacagg GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

## JC90

cgatgatgaagatccccaccnaa CCGAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

## Bgl II

## Sfi I

## Sma I

## Bam H I

ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GAG GCC CCG GGG ATC CGA ATT

## Nco I

## Xho I

## Bgl II

CGA GCT CGA CTA GCT AGC TGA CTC GAG AGA TCT ATGAAT

## Sac I

cgtagatactgaaaaacccc GCAAGTT cacttcaactgtgcatgtg caccatcctaattc

162

ABS2

53

ABS1 5' CGTTTGGAACTACTACAGG 3'

JC90 5' CGATGATGAAGATACCCACCAAA 3'

162 5' GGGGTTTTTCAGTATCTACG 3'

ABS2 5' CACGATGCACAGTTGAAGTG 3'

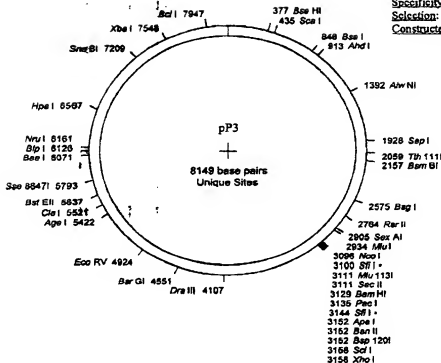
53 5' GAAATTGAGATGGTGACGATCCAC 3'

FIGURE 7





pP3



Application: 2HY (prey)  
 Backbone: pACT11st  
 Specificity: Sfi oriented  
 Selection: ampicillin  
 Constructed by: SW

ABS1

CG cggttggaatcactacagg GATGTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90

Bgl II

cgatgatgaagataccccaccaga CCCAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

Sfi I

Sac II

ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GCC GGA CGG GCC GCG GCC GCA

Neo I

CTA GTG GGG ATC CTT AAT TAA GGG CCA CTG GGG CCC CTC GAG AGA TCT

Stop

ATGAAT cgtagatactgaaaccccc GCAAAGTT cacttcacatgcatcgtg caccatctcaattc

162

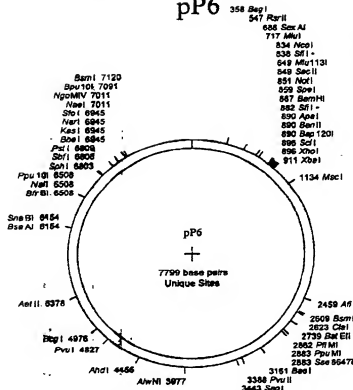
ABS2

53

ABS1 5' CGTTTGAATCACTACAGG 3'  
 JC90 5' CGATGATGAAGATACCCACCAAA 3'  
 162 5' GGGGTTTTTCAGTATCTACG 3'  
 ABS2 5' CACGATGCACAGTTGAAGTG 3'  
 53 5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 9

pP6<sup>10</sup>



**Alias:** pGAD3S2XNS1  
**Application:** 2HY (prey)  
**Backbone:** pGAD3S2X  
**Specificity:** Sfi non-oriented  
**Selection:** ampicillin  
**Constructed by:** SW

## ABS1

cgtttggaatcactacagg

GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90

cgatgatgaagataccccaccaa

CCCAAAAAGAGATCCTAGAACTA

## ST I

**Sac II**

**Spe 1**

## Bam HI

GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C

**Neo I**

**Not I**

•

**STOP**

**SG I**

## ho I

## Хбѣ І

TT AAT TAA GGG CCA CTG GGG CCC CTC GAG TAG CTA GGG TCT AGA  
STOP STOP STOP

GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCAGTGGCCG TCGTTTTA

CAACGTCGTGACTGGGAAAACCCTGATCTATGAAT cgtagataactgaaaaacccc GCAA

GTT	cacticaactgtgcatgtg	caccatctcaattctttc
-----	---------------------	--------------------

162

**ABS2**

53

**ABS1 5' CGTTTGGGAATCACTACAGG 3'**

JC90 5' CGATGATGAAGATACCCCACCAA 3'

162 5' GGGGTTTTTTCAGTATCTACG 3'

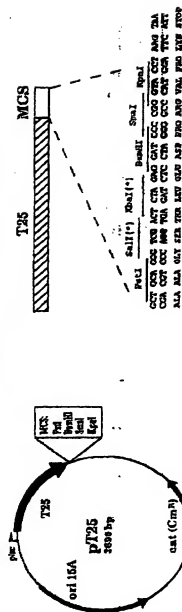
ABS2 5' CACGATGCACAGTTGAAGTG 3'

53      5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 10

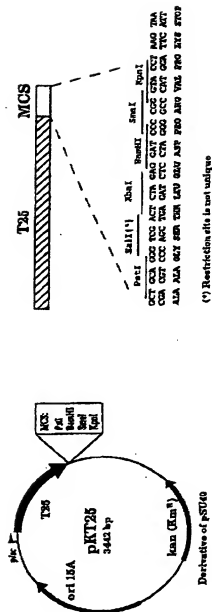


# VECTORS EXPRESSING THE T25 FRAGMENT



Derivative of pUTG184

(\*) Restriction sites are not unique



Derivative of pSU60

(\*) Restriction sites are not unique

FIGURE 12

13

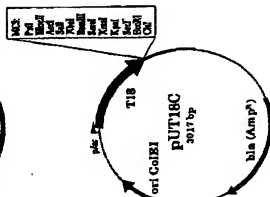
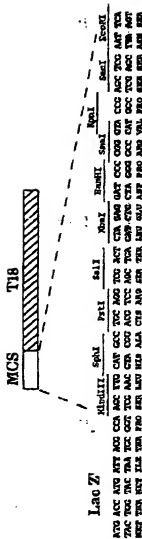
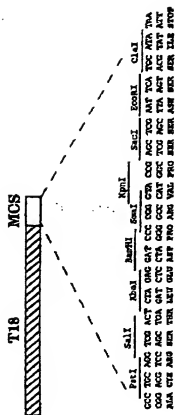
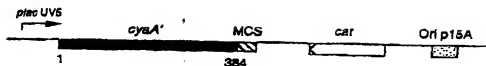


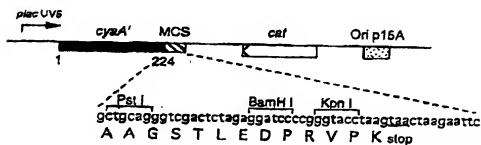
FIGURE 13



## pCmAHL1



## pT25



## pT18

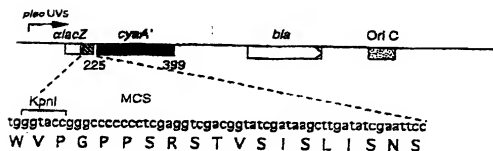


FIGURE 14

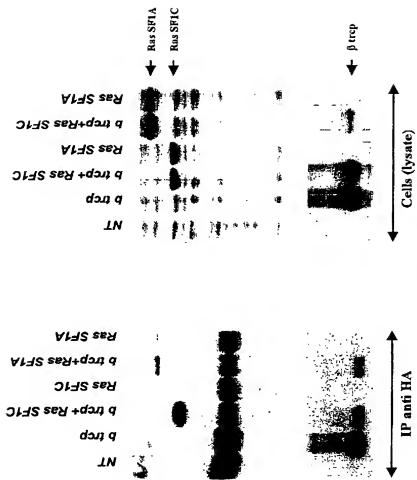
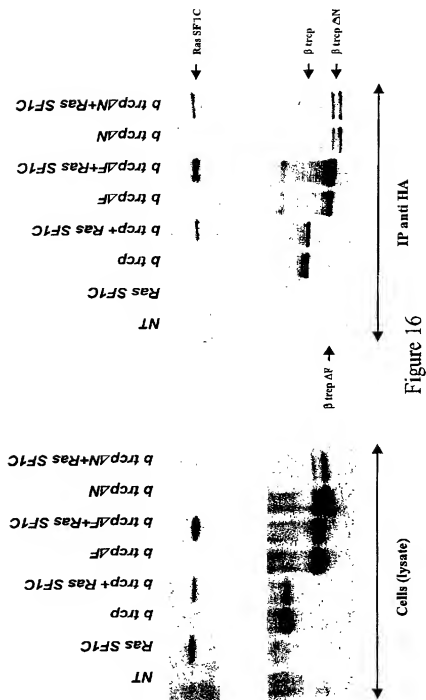


Figure 15





HATrCPWT	+	-	-	-	-
HATrCP1-260	-	+	-	-	-
HATrCP1-333	-	-	+	-	-
HATrCP261-569	-	-	-	+	-
RasSFIC-Myc	+	+	+	+	+

IP $\alpha$ -HA  
WB $\alpha$ -Myc-HRP



17/20

Total lysates  
WB $\alpha$ -Myc-HRP



IP $\alpha$ -HA  
WB $\alpha$ -HA

— 83kD  
— 62kD  
— 47,5kD  
— 32,5kD  
— 25kD

HATrCPWT →

HATrCP261-569 →

HATrCP1-333 →

HATrCP1-260 →



Figure 17

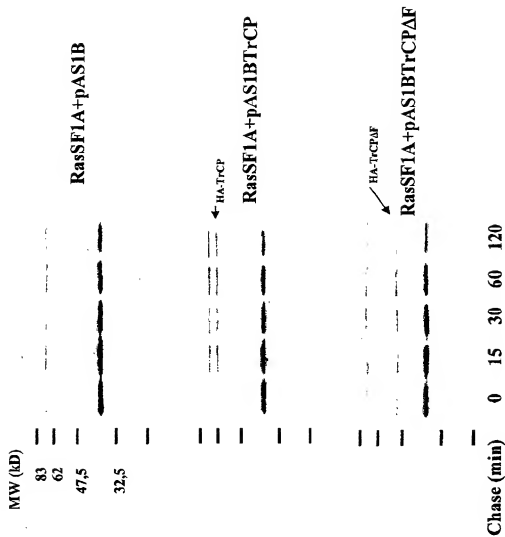


Figure 18

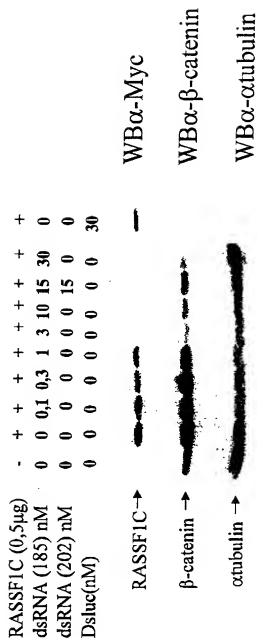


Figure 19

Interaction  
RasSFI

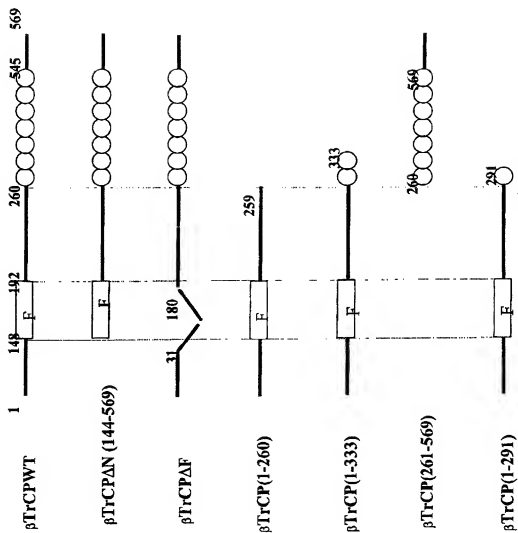


Figure 20